Clinica Chimica Acta 427 (2014) 15–20

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Invited critical review

Serum free light chains in clinical laboratory diagnostics

Ellen Jenner *

The Binding Site Group Ltd., 8 Calthorpe Road, Edgbaston, Birmingham B15 1QT, UK

ARTICLE INFO

Article history:
Received 6 June 2013
Received in revised form 22 August 2013
Accepted 23 August 2013
Available online 30 August 2013

Keywords:
Free light chains
Multiple myeloma
AL amyloidosis
Immunoassays
Serum protein
Electrophoresis

ABSTRACT

Monoclonal free light chains (FLCs) are important disease biomarkers in patients with plasma cell-proliferative disorders. The increasing evidence for clonal diversity and evolution in multiple myeloma highlights the importance of laboratory algorithms that measure both intact immunoglobulins and monoclonal FLCs, at diagnosis and when monitoring response to treatment. A particular focus in the field has been on the utility of serum FLC (sFLC) assays to replace urine electrophoresis for monoclonal FLC measurement. Due to the limited sensitivity and practical constraints of urine analysis, a serum-based algorithm of SPE and sFLC has been adopted by many laboratories as a first line screen in patients with suspected monoclonal gammopathies. This review will discuss the data supporting the use of this simple serum-based algorithm at initial diagnosis, including its utility for the rapid identification of monoclonal FLC in the setting of unexplained acute kidney injury, and provide a comprehensive review of the diagnostic sensitivity of sFLC in patients with multiple myeloma, AL amyloidosis and light chain deposition disease.

Contents

1. Introduction

Monoclonal immunoglobulin free light chains (FLC) are an important diagnostic marker for monoclonal gammopathies and, for more than 150 years, the presence of Bence Jones protein (FLCs) in the urine has been the key indicator of monoclonal FLC production. However, during the last decade, there has been a paradigm shift with the availability of automated immunoassays that independently measure kappa (κ) and lambda (λ) FLC in the serum (Freelite®).

Serum concentrations of FLCs are dependent upon the balance between production and renal clearance. Serum FLCs (sFLCs) are rapidly cleared through the renal glomeruli with half-lives of between 2 and

Abbreviations: sFLC, serum free light chain; κ, kappa; λ, lambda; sFLC κ/λ ratio, ratio of κ sFLC to λ sFLC concentration; MM, multiple myeloma; LCMM, light chain MM; IIMM, intact immunoglobulin MM; NSMM, nonsecretory MM; LCDD, light chain deposition disease; MGUS, monoclonal gammopathy of undetermined significance; WM, Waldenström's macroglobulinaemia; SPE, serum protein electrophoresis; UPE, urine protein electrophoresis; IFE, immunofixation electrophoresis; Mabs, monoclonal antibodies; AKI, acute kidney injury.

* Tel.: +44 121 456 9500; fax: +44 121 456 9749.
E-mail address: ellen.jenner@bindingsite.co.uk.

0009-8981
http://dx.doi.org/10.1016/j.cca.2013.08.018

6 -h before being metabolised in the proximal tubules of the nephrons. Under normal circumstances, little protein escapes to the urine so sFLC concentrations are a more accurate representation of production levels. When there is increased polyclonal immunoglobulin production and/or renal impairment, both κ and λ sFLC concentrations can increase 30- to 40-fold. However, the relative concentrations of κ to λ (i.e., the κ/λ ratio) remain unchanged, or only slightly increase (Section 2.2). In contrast, the production of a monoclonal excess of one FLC type in patients with a plasma cell dyscrasia gives an abnormal serum κ/λ ratio, providing a numerical indicator of clonality.

The clinical importance of sFLC assays for monoclonal gammopathies is now well established, and their utility for the diagnosis, prognosis and monitoring of these patients is acknowledged in national and international guidelines [1–7]. This article will discuss the different laboratory methods for the measurement of FLC and provide a comprehensive review of the published data supporting the application of sFLC analysis at diagnosis of monoclonal gammopathies, including the use of sFLC assays to screen for nephrotoxic FLC in the setting of myeloma kidney.

2. Laboratory methods for the detection of monoclonal FLC

Laboratory methods to screen for monoclonal gammopathies historically comprise serum protein electrophoresis (SPE) and urine protein electrophoresis (UPE). Monoclonal proteins (M-proteins) migrate as discrete bands on an electrophoretic gel, appearing as a peak on a densitometric trace, which provides a semi-quantitative value for the amount of M-protein. Following the identification of an M-protein by SPE, serum immunofixation electrophoresis (sIFE) is required for confirmation of clonality and subsequent typing. With an analytical sensitivity of between 500 and 2000 mg/L [8], a major limitation of SPE is the inability to detect low-level monoclonal proteins, particularly FLCs. Serum IFE is approximately 10-fold more sensitive and may pick up additional monoclonal proteins that are undetected by SPE. However, the serum of patients with oligosecretory diseases, such as light chain multiple myeloma (LCMM), AL-amyloidosis and light chain deposition disease (LCDD) often does not contain monoclonal FLCs at a level sufficient to be detected by either SPE or sIFE [9–11].

For over 150 years, monoclonal FLCs in the urine (Bence Jones protein; BJP) have been an important diagnostic marker for multiple myeloma (MM). UPE and urine IFE (uIFE) are more sensitive than serum electrophoresis techniques for detecting monoclonal FLCs, and FLCs can be detected in the urine at less than 20 mg/L, although most laboratories claim an FLC detection limit of 40–50 mg/L.

Despite the additional sensitivity offered by UPE and uIFE, these techniques are not without their technical and practical limitations. First, FLC levels in the serum must increase significantly before the proximal tubular re-absorptive mechanisms are overwhelmed and the FLCs appear in the urine. Nowrousian et al. [12] reported that the median levels of monoclonal κ and λ FLC required in the serum before Bence Jones proteinuria occurred were 113 mg/L and 278 mg/L, respectively. Therefore, low-level monoclonal FLCs in the serum may not be detected in the urine, and urine BJP tests are not a direct reflection of the underlying monoclonal FLC production rate. The second important consideration for routine clinical practice is the delay associated with obtaining a urine BJP result. The early detection of monoclonal FLC facilitates the prompt diagnosis and timely initiation of treatment to improve clinical outcome; however, delays can occur due to poor urine compliance, the requirement for 24-h urine collection and batch testing of urine samples by the laboratory. Studies have reported variable urine compliance values of between 5% and 59% [13–17]. The third potential limitation of urine based algorithms for the identification of monoclonal FLCs is the subjective interpretation of electrophoresis results. The detection of low-level monoclonal FLCs is particularly problematic. 'Ladder banding' in concentrated urine samples may give the false impression of monoclonality, and heavy proteinuria containing polyclonal FLCs may give high background staining hindering accurate interpretation. Due to the highlighted limitations of urinalysis, international guidelines now recommend that serum FLC testing replaces urine electrophoresis in the diagnosis of monoclonal gammopathies [7].

2.1. Immunoassays for serum monoclonal FLC testing

In 2001, the availability of automated sFLC immunoassays (Freelite, Binding Site, UK) has enabled routine and sensitive laboratory quantification of monoclonal FLC in the serum. The sFLC tests provide an independent measurement of κ and λ FLC, and the calculation of a κ/λ sFLC ratio provides a sensitive numerical indicator of clonality. In patients with plasma cell dyscrasias, the excess clonal production of only one FLC type, frequently with bone marrow suppression of the alternate FLC, leads to often highly abnormal κ/λ ratios. The assays are latex-enhanced immunoassays and allow measurement of FLC concentrations as low as 1.5 mg/L and 3 mg/L for κ and λ FLCs, respectively, far below normal serum concentrations [18]. The sFLC assays should not be confused with total κ and λ light chain (TLC) assays, which detect all forms of κ or λ light chains (FLC plus those that form intact immunoglobulins). TLC assays are insensitive for the detection of sFLC [19] and are not recommended by international guidelines.

It is essential that sFLC immunoassays utilise antibodies that have high specificity and affinity. For assays detecting homogeneous antigens, monoclonal antibodies (Mabs) are very successful. However, a reliable recognition of a full range of monoclonal FLC is hampered due to the heterogeneity of FLC molecules, arising from a combination of genetic recombination, allotypic and isotypic variation and somatic hypermutation of the variable regions after antigen exposure. As a Mab will only recognise a single epitope, individual Mabs will be unable to detect the diverse range of monoclonal FLC produced by patients with monoclonal gammopathies. Hence, the Freelite assays were developed using polyclonal antibodies, raised in sheep. These assays can recognise a wide variety of FLC epitopes, including the diverse range of pathological monoclonal FLC produced by patients with monoclonal gammopathies.

Several groups have developed Mab-based FLC immunoassays [20–25]. A large number of clinical studies are still required to assess their clinical utility, although published data has failed to demonstrate their clinical equivalence to the established polyclonal assays [26,27]. International guidelines for sFLC measurement are based on results obtained with Freelite, and the data discussed in this review has been obtained using these polyclonal assays.

2.2. Interpretation of sFLC assay results at diagnosis

For sFLC analysis, both κ and λ FLCs should be measured and κ/λ ratios calculated. Results are considered abnormal when they are outside published normal ranges [18] (serum κ FLC: 3.3–19.4 mg/L; serum λ FLC: 5.7–26.3 mg/L; serum κ/λ ratio: 0.26–1.65). If serum κ FLC, λ FLC and κ/λ ratio are all within the normal ranges, and accompanying serum electrophoretic tests are normal it is unlikely that the patient has a monoclonal gammopathy. Conversely, abnormal κ/λ ratios, along with an increase in either κ or λ FLC, support the diagnosis of a monoclonal gammopathy and require further investigation, although borderline results require careful interpretation. Borderline abnormal κ/λ ratios can occasionally be seen in patients with polyclonal increases in FLC, such as in patients with renal impairment, and in patients with polyclonal hypergammaglobulinaemia, caused by for example infectious or inflammatory disorders [28,29]. This highlights the importance of considering additional clinical and laboratory parameters when interpreting sFLC results.

3. FLC incorporation into laboratory diagnostic panels for monoclonal gammopathies

The utility of sFLC assays at diagnosis has been documented for a broad spectrum of monoclonal plasma cell disorders, from the

asymptomatic disorders monoclonal gammopathy of undetermined significance (MGUS) and smouldering multiple myeloma (SMM) to the symptomatic disorders multiple myeloma (MM), Waldenström's macroglobulinaemia (WM), AL amyloidosis, LCDD, solitary plasmacytoma and POEMS. Numerous studies have evaluated the use of sFLC assays alongside other laboratory tests as part of the initial diagnostic screen for patients with a suspected monoclonal gammopathy [13,15,17,28–33].

The most extensive study included 1877 patients (467 MM, 191 SMM, 524 MGUS, 29 plasmacytoma, 26 WM, 581 AL amyloidosis, 18 LCDD and 31 POEMS) who had a complete panel of SPE, sIFE, sFLC, UPE and uIFE results obtained within 30 days of diagnosis [33]. When comparing the combination of SPE and sFLC with SPE, sFLC and sIFE, 58 patients had a negative screen (44 MGUS, 7 POEMS, 5 AL amyloidosis, 1 plasmacytoma and 1 SMM). However, all MM, WM and LCDD patients were detected. Furthermore, the addition of the sFLC assays identified 30 patients (23 AL amyloidosis, 6 MM and 1 LCDD) that were not detected by the traditional panel of serum and urine tests. The omission of urine analysis missed just 23 patients (15 MGUS, 1 extramedullary myeloma, 1 LCDD and 6 AL amyloidosis). As part of their conclusion, Katzmann et al. stated that due to the small incremental sensitivity provided by urine studies and sIFE, the use of SPE with sFLC analysis provides a simple and efficient initial diagnostic screen for high tumour burden monoclonal gammopathies. They observed that urine studies and sIFE may be ordered more selectively.

Based on the available published data, the International Myeloma Working Group (IMWG) conclude that in the context of screening for the presence of MM or related disorders, the sFLC assay in combination with SPE and sIFE yields high sensitivity and negates the requirement for 24-h urine studies for diagnosis other than for AL amyloidosis [7]. In their most recent guidelines, the IMWG recommend sFLC analysis as part of the standard investigative workup in newly diagnosed patients with plasma cell dyscrasias [6], a recommendation advocated in guidelines published by the US National Comprehensive Cancer Network (NCCN) [1]. This also provides valuable prognostic information; the prognostic utility of sFLC concentrations and ratios at disease presentation and following treatment has been demonstrated by a number of groups [34–37]. International guidelines recommend that the sFLC assay is performed at diagnosis as a prognostic marker for all patients with MM, as well as for patients with MGUS, SMM, solitary plasmacytoma and AL amyloidosis [7].

4. Diagnostic sensitivity of sFLC assays for monoclonal gammopathies

The frequency of abnormal sFLC κ/λ ratios that can be expected in patients with MM (including light chain MM (LCMM), nonsecretory MM (NSMM) and intact immunglobulin MM (IIMM)) and AL amyloidosis is shown in Fig. 1.

4.1. Light chain multiple myeloma

LCMM accounts for around 20% of all cases of MM. Its clinical diagnosis is confirmed by the presence of monoclonal FLCs in the serum or urine, in the absence of intact monoclonal immunoglobulins, alongside clonal bone marrow plasma cells and the presence of end organ damage. Screening with SPE alone may fail to detect over 40% of cases of LCMM [38], and considering the limitations associated with detecting monoclonal FLC in the urine, sFLC analysis forms an important part of the diagnostic algorithm for these patients. All 224 patients with LCMM in a study by Bradwell et al. [39] had abnormal concentrations of the appropriate sFLC and abnormal κ/λ ratios at the time of diagnosis (Fig. 1). The same 100% diagnostic sensitivity of sFLC ratios for LCMM has been found in numerous studies, which, in total, have included over 600 patients [12,16,28,38–45].

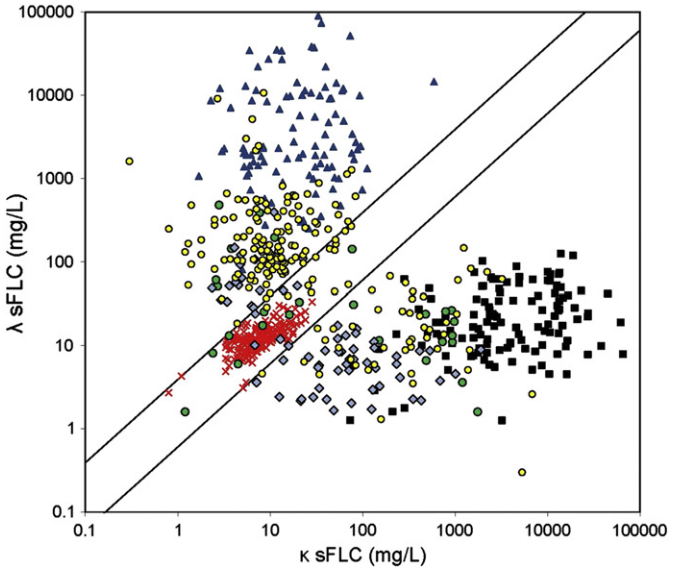

Fig. 1. Dotplot of sFLC concentrations in patients with monoclonal gammopathies. The normal 100% reference range for the sFLC κ/λ ratio (0.26–1.65) is represented by the diagonal lines. Serum samples with abnormal κ/λ ratios lie outside of these lines. Normal sera: red crosses; κ LCMM: black squares; λ LCMM: blue triangles; NSMM: green circles; IIMM: blue diamonds; AL amyloidosis: yellow circles. Data adapted from [11,39,51].

4.2. Nonsecretory multiple myeloma

Accounting for 1–5% of all MM patients, NSMM is characterised by the absence of monoclonal proteins in serum and urine by IFE [9,10,46,47]. However, some NSMM patients produce monoclonal immunoglobulins that, despite being undetected in the serum, can be detected immunohistochemically in bone marrow plasma cells, and it is considered that only 10–15% of NSMM patients are true 'non-producers' [48], in whom tumour plasma cells contain no detectable immunoglobulins.

The sensitivity of sFLC immunoassays has proved particularly beneficial for detecting monoclonal FLCs in patients previously deemed nonsecretory according to electrophoresis. In a study of 28 NSMM patients [10], 68% had elevated κ or λ sFLC concentrations and abnormal κ/λ ratios (Fig. 1). Surprisingly, in 9 of the 28 patients, no monoclonal bands were seen by IFE even though the immunoassays indicated sFLC concentrations of >200 mg/L, greater than the detection limit of IFE. Further investigation of 2 samples (with 931 mg/L and 978 mg/L monoclonal κFLC) revealed polymerised FLC, which act as multi-antigenic targets in immunoassays, leading to overestimation of antigen concentrations, and could also account for their absence or diffuse appearance by IFE.

Measurement of sFLC at diagnosis for many NSMM patients has produced clear benefits for the patient, avoiding diagnostic delays [49], and sFLC analysis is therefore recommended by the IMWG for patients with NSMM [7].

4.3. Intact immunoglobulin multiple myeloma

IIMM comprises around 80% of all cases of MM. Since this type of MM is characterised by the secretion of monoclonal intact immunoglobulins, SPE and sIFE play an essential role in the diagnosis of these patients. However, in around 95% of patients with IIMM monoclonal FLCs are also secreted [33,36,50–52]. In these patients, the utility of sFLC analysis lies in disease monitoring and patient prognosis. The short serum half-life of FLCs make them useful markers of clonal disease and the monitoring of monoclonal sFLC levels can provide a more accurate assessment of the rate of treatment responses than that provided

by intact immunoglobulins, whose half-lives are considerably longer [53].

Importantly, the serological diagnosis of disease relapse in IIMM patients cannot be relied upon by measuring monoclonal intact immunoglobulins alone. Clonal evolution in MM is increasingly being recognised [2,54–57] and is associated with a change in the monoclonal protein(s) being produced. For example, patients with IIMM at diagnosis may relapse with monoclonal FLCs only, a phenomenon termed light chain escape [58,59] (Fig. 2). Due to high monoclonal FLC concentrations, these patients are prone to renal complications, and regular assessment of sFLCs during patient monitoring can provide early indications of renal impairment and any risk of renal failure. In light of the complex clonal dynamics involved in MM, it is advisable that both monoclonal intact immunoglobulins and sFLCs are measured. Interestingly, one study demonstrated that the monoclonal protein type in patients with LCMM does not alter at disease relapse [59] (Fig. 2).

4.4. AL amyloidosis and light chain deposition disease

AL amyloidosis and LCDD are two disorders caused by the extracellular precipitation of monoclonal light chains which disrupt the structure and function of multiple organs. In AL amyloidosis, light chain fragments, most commonly λ type, accumulate as insoluble amyloid fibrils and frequently affect the kidney and heart. The monoclonal light chains most often involved in LCDD are κ type and are precipitated on the basement membranes of cells in the kidneys and various other organs. While a definitive diagnosis of AL amyloidosis or LCDD is established by a tissue biopsy and histological examination, the detection of the monoclonal protein provides important supportive evidence of an underlying plasma cell dyscrasia.

Studies evaluating the diagnostic performance of the sFLC ratio in AL amyloidosis have documented a diagnostic sensitivity ranging from 75% to 98% [11,33,60–64]. The observation that an abnormal sFLC ratio has a greater diagnostic sensitivity than the combination of serum or urine IFE (98% vs. 79%) [11] has been supported by some studies [60] but not by others [61–63]. However, in all published studies to date, sFLC analysis has proven to be an important complementary technique to electrophoresis, which is reflected in IMWG guidelines that recommend sFLC analysis alongside sIFE and uIFE to screen patients with suspected AL amyloidosis [7]. Abnormal sFLC ratios are also found in 88–100% of patients with LCDD, of which approximately 1/3 of patients are negative by sIFE [33,65,66]. As such, sFLC analysis is a useful addition to electrophoretic tests when screening for and monitoring LCDD and is recommended by the IMWG [7].

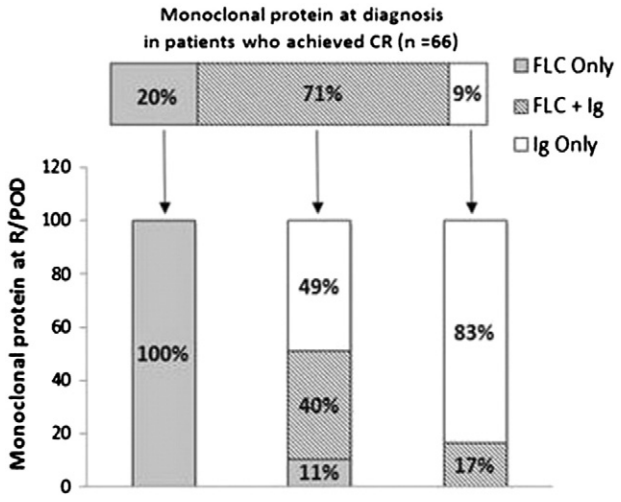

Fig. 2. Changes in monoclonal protein type at relapse or progression of disease (R/POD). Reprinted with permission from Macmillan Publishers Ltd, nature.com [59].

5. Serum FLC assays for early detection of myeloma kidney

The clonal production of nephrotoxic FLCs is the predominant cause of the renal injury associated with MM and related monoclonal gammopathies. Monoclonal FLCs can induce a number of different pathologies within the kidney, which in turn can result in different clinical presentations. Of these, the most common presentation of FLC-induced renal injury is acute kidney injury (AKI) secondary to the tubular interstitial pathology cast nephropathy. In cast nephropathy, also known as myeloma kidney, waxy casts form in the distal tubules of the nephron due to the aggregation of FLC with Tamm Horsfall protein, causing interstitial inflammation and fibrosis. Found in approximately 90% of dialysis-dependent MM patients [16], myeloma kidney is the most common cause of AKI in MM.

A timely diagnosis is critically important in order to initiate treatment regimens to rapidly reduce FLC concentrations in patients with myeloma kidney. Such early FLC reductions are associated with improved survival [67]. Therefore, a screening algorithm designed to enable the rapid diagnosis of myeloma kidney as the cause of AKI has recently been proposed by the International Kidney and Monoclonal Gammopathy Research Group [68]. This algorithm proposes that the first priority in assessing a patient with unexplained AKI is to determine if monoclonal FLCs are present. To enable this, either sFLC assays or uIFE can be utilised, but given the limitations of urine analyses described in Section 2, many laboratories now use sFLC assays first line. When monoclonal FLCs are present, the serum concentration of the involved FLCs can provide significant guidance as to the likelihood of myeloma kidney as the renal pathology. Patients with myeloma kidney have baseline monoclonal sFLC concentrations that are typically above 500 mg/L [16,67,69–74], and a cutoff of 500 mg/L is commonly used as the level above which cast nephropathy is the likely pathology. A small number of patients with a high concentration of monoclonal FLC may have an alternative diagnosis to cast nephropathy [75] and so a renal biopsy should be considered for confirmation.

6. Conclusion

The incorporation of sFLC assays into diagnostic algorithms for plasma cell disorders had led to a paradigm shift in the understanding of these diseases. There has been a particular focus on the utility of sFLC analysis in AL amyloidosis, LCMM and NSMM, and recently for the rapid identification of myeloma kidney as a cause of unexplained AKI. As it becomes increasingly understood that MM is a multi-clonal disease, moving forward it is likely that serum-based tests for the measurement of monoclonal FLCs will become a greater adjunct to traditional measurements to identify all clones and monitor clonal evolution in patients with MM.

Acknowledgements

The author would like to thank Josie Evans, Kelly Endean, Richard Hughes and Stephen Harding for their assistance in preparing and proof reading the manuscript.

References

[1] Anderson KC, Alsina M, Bensinger W, et al. NCCN Clinical practice guidelines in oncology for multiple myeloma. J Natl Compr Canc Netw 2011;9:1146–83.
[2] Bahlis NJ. Darwinian evolution and tiding clones in multiple myeloma. Blood 2012;120:927–8.
[3] Bird J, Behrens J, Westin J, et al. UK Myeloma Forum (UKMF) and Nordic Myeloma Study Group (NMSG): guidelines for the investigation of newly detected M-proteINS and the management of monoclonal gammopathy of undetermined significance (MGUS). Br J Haematol 2009;147:22–42.
[4] Bird JM, Cavenagh J, Samson D, Mehta A, Hawkins P, Lachmann H. Guidelines on the diagnosis and management of AL amyloidosis. Br J Haematol 2004;125:681–700.
[5] Bird JM, Owen RG, D'sa S, et al. Guidelines for the diagnosis and management of multiple myeloma 2011. Br J Haematol 2011;154:32–75.

[6] Dimopoulos M, Kyle R, Fermand JP, et al. Consensus recommendations for standard investigative workup: report of the International Myeloma Workshop Consensus Panel 3. Blood 2011;117:4701–5.
[7] Dispenzieri A, Kyle R, Merlini G, et al. International Myeloma Working Group guidelines for serum-free light chain analysis in multiple myeloma and related disorders. Leukemia 2009;23:215–24.
[8] Bradwell AR, Carr-Smith HD, Mead GP, et al. Highly sensitive, automated immunoassay for immunoglobulin free light chains in serum and urine. Clin Chem 2001;47:673–80.
[9] Blade J, Kyle RA. Nonsecretory myeloma, immunoglobulin D myeloma and plasma cell leukemia. Hematol Oncol Clin North Am 1999;13:1259–72.
[10] Drayson M, Tang LX, Drew R, Mead GP, Carr-Smith H, Bradwell AR. Serum free light-chain measurements for identifying and monitoring patients with nonsecretory multiple myeloma. Blood 2001;97:2900–2.
[11] Lachmann HJ, Gallimore R, Gillmore JD, et al. Outcome in systemic AL amyloidosis in relation to changes in concentration of circulating free immunoglobulin light chains following chemotherapy. Br J Haematol 2003;122:78–84.
[12] Nowrousian MR, Brandhorst D, Sammet C, et al. Serum free light chain analysis and urine immunofixation electrophoresis in patients with multiple myeloma. Clin Cancer Res 2005;11:8706–14.
[13] Hill PG, Forsyth JM, Rai B, Mayne S. Serum free light chains: an alternative to the urine Bence Jones proteins screening test for monoclonal gammopathies. Clin Chem 2006;52:1743–8.
[14] Holding S, Spradbery D, Robson EJD, Dore PC, Wilmot R, Shields ML. Combination of serum free light chain analysis with capillary zone electrophoresis improves screening for monoclonal gammopathies. Blood 2007;110:1497a.
[15] Holding S, Spradbery D, Hoole R, et al. Use of serum free light chain analysis and urine protein electrophoresis for detection of monoclonal gammopathies. Clin Chem Lab Med 2011;49:83–8.
[16] Hutchison CA, Plant T, Drayson M, et al. Serum free light chain measurement aids the diagnosis of myeloma in patients with severe renal failure. BMC Nephrol 2008;9:11.
[17] Robson EJD, Taylor J, Beardsmore C, Basu S, Mead G, Lovatt T. Utility of serum free light chain analysis when screening for lymphoproliferative disorders. Lab Med 2009;40:325–9.
[18] Katzmann JA, Clark RJ, Abraham RS, et al. Serum reference intervals and diagnostic ranges for free kappa and free lambda immunoglobulin light chains: relative sensitivity for detection of monoclonal light chains. Clin Chem 2002;48:1437–44.
[19] Marien G, Oris E, Bradwell AR, Blanckaert N, Bossuyt X. Detection of monoclonal proteins in sera by capillary zone electrophoresis and free light chain measurements. Clin Chem 2002;48:1600–1.
[20] Davern S, Tang LX, Williams TK, et al. Immunodiagnostic capabilities of anti-free immunoglobulin light chain monoclonal antibodies. Am J Clin Pathol 2008;130:702–11.
[21] Campbell JP, Cobbold M, Wang Y, et al. Development of a highly-sensitive multi-plex assay using monoclonal antibodies for the simultaneous measurement of kappa and lambda immunoglobulin free light chains in serum and urine. J Immunol Methods 2013;391:1–13.
[22] Nakano T, Nagata A. ELISAs for free human immunoglobulin light chains in serum: improvement of assay specificity by using two specific antibodies in a sandwich detection method. J Immunol Methods 2004;293:183–9.
[23] Ricotta D, Radeghieri A, Amoroso B, Caimi L. Serum free light chains in MM: comparison of 2 assays. Haematologica 2007;92:PO1018a.
[24] Vávrová J, Friedecký B, Tichý M, Holecková M, Maisnar V, Hájek R. The determination of free light chains by immunoturbidimetry and ELISA. Klin Biochem Metab 2008;16:97–101.
[25] Velthuis HT, Knop I, Stam P, et al. N Latex FLC—new monoclonal high-performance assays for the determination of free light chain kappa and lambda. Clin Chem Lab Med 2011;49:1323–32.
[26] Drayson MT, Carr Smith HD. Clinical comparison of new monoclonal antibody-based nephelometric assays for free light chain kappa and lambda to polyclonal antibody-based assays and immunofixation electrophoresis. Clin Chem Lab Med 2012;50:587–8.
[27] Lock R, Saleem R, Roberts E, et al. A multicentre study comparing two methods for serum free light chain analysis. Ann Clin Biochem 2013;50:255–61.
[28] Piehler AP, Gulbrandsen N, Kierulf P, Urdal P. Quantitation of serum free light chains in combination with protein electrophoresis and clinical information for diagnosing multiple myeloma in a general hospital population. Clin Chem 2008;54:1823–30.
[29] Vermeersch P, Van Hoovels L, Delforge M, Marien G, Bossuyt X. Diagnostic performance of serum free light chain measurement in patients suspected of a monoclonal B-cell disorder. Br J Haematol 2008;143:496–502.
[30] Abadie JM, Bankson DD. Assessment of serum free light chain assays for plasma cell disorder screening in a Veterans Affairs population. Ann Clin Lab Sci 2006;36:157–62.
[31] Augustson BM, Katsavara H, Reid SD, Mead GP, Shirfield M, Bradwell AR. Monoclonal gammopathy screening: improved sensitivity using the serum free light chain assay. Haematologica 2005;90:PO1302a.
[32] Bakshi NA, Gulbranson R, Garstka D, Bradwell AR, Keren DF. Serum free light chain (FLC) measurement can aid capillary zone electrophoresis in detecting subtle FLC-producing M proteins. Am J Clin Pathol 2005;124:214–8.
[33] Katzmann JA, Kyle RA, Benson J, et al. Screening panels for detection of monoclonal gammopathies. Clin Chem 2009;55:1517–22.
[34] Kyrtsonis MC, Vassilakopoulos TP, Kafasi N, et al. Prognostic value of serum free light chain ratio at diagnosis in multiple myeloma. Br J Haematol 2007;137:240–3.
[35] Iwama KI, Chihara D, Tsuda K, et al. Normalization of free light chain kappa/lambda ratio is a robust prognostic indicator of favorable outcome in patients with multiple myeloma. Eur J Haematol 2012;90:134–41.
[36] Snozek CL, Katzmann JA, Kyle RA, et al. Prognostic value of the serum free light chain ratio in newly diagnosed myeloma: proposed incorporation into the international staging system. Leukemia 2008;22:1933–7.
[37] Sthaneshwar P, Nadarajan V, Maniam JA, Nordin N, Gin Gin G. Serum free light chains: diagnostic and prognostic value in multiple myeloma. Clin Chem Lab Med 2009;47:1101–7.
[38] Abraham RS, Clark RJ, Bryant SC, et al. Correlation of serum immunoglobulin free light chain quantification with urinary Bence Jones protein in light chain myeloma. Clin Chem 2002;48:655–7.
[39] Bradwell AR, Carr-Smith HD, Mead GP, Harvey TC, Drayson MT. Serum test for assessment of patients with Bence Jones myeloma. Lancet 2003;361:489–91.
[40] Drayson MT, Morgan GJ, Jackson GH, et al. Prospective study of serum FLC and other M-protein assays: when and how to measure response? Clin Lymphoma Myeloma 2009;9:A346a.
[41] Harding SJ, Mead GP, Bradwell AR, Berard AM. Serum free light chain immunoassay as an adjunct to serum protein electrophoresis and immunofixation electrophoresis in the detection of multiple myeloma and other B-cell malignancies. Clin Chem Lab Med 2009;47:302–4.
[42] Kang SY, Suh JT, Lee HJ, Yoon HJ, Lee WI. Clinical usefulness of free light chain concentration as a tumor marker in multiple myeloma. Ann Hematol 2005;84:588–93.
[43] Mösbauer U, Ayuk F, Schieder H, Lioznov M, Zander AR, Kroger N. Monitoring serum free light chains in patients with multiple myeloma who achieved negative immunofixation after allogeneic stem cell transplantation. Haematologica 2007;92:275–6.
[44] van Rhee F, Bolejack V, Hollmig K, et al. High serum-free light chain levels and their rapid reduction in response to therapy define an aggressive multiple myeloma subtype with poor prognosis. Blood 2007;110:827–32.
[45] Wolff F, Thiry C, Willems D. Assessment of the analytical performance and the sensitivity of serum free light chains immunoassay in patients with monoclonal gammopathy. Clin Biochem 2007;40:351–4.
[46] Abdalla IA, Tabbara IA. Nonsecretory multiple myeloma. South Med J 2002;95:761–4.
[47] Dreicer R, Alexanian R. Nonsecretory multiple myeloma. Am J Hematol 1982;13:313–8.
[48] Raubenheimer EJ, Dauth J, Senekal JC. Non-secretory IgA kappa myeloma with distended endoplasmic reticulum: a case report. Histopathology 1991;19:380–2.
[49] Middela S, Kanse P. Nonsecretory multiple myeloma. Indian J Orthop 2009;43:408–11.
[50] Dispenzieri A, Zhang L, Katzmann JA, et al. Appraisal of immunoglobulin free light chain as a marker of response. Blood 2008;111:4908–15.
[51] Mead GP, Carr-Smith HD, Drayson MT, Morgan GJ, Child JA, Bradwell AR. Serum free light chains for monitoring multiple myeloma. Br J Haematol 2004;126:348–54.
[52] Owen RG, Child JA, Rawstron AC, et al. Defining complete response in multiple myeloma: role of the serum free light chain assay and multiparameter flow cytometry. Blood 2007;110:1479a.
[53] Mead GP, Carr-Smith HD, Galvin G, Pratt G, Bradwell AR. Efficacy of high dose treatment and response to individual chemotherapy agents in myeloma is indicated by changes in serum free light chain concentrations. Clin Chem 2004;50:C20a.
[54] Egan JB, Shi CX, Tembe W, et al. Whole-genome sequencing of multiple myeloma from diagnosis to plasma cell leukemia reveals genomic initiating events, evolution, and clonal tides. Blood 2012;120:1060–6.
[55] Walker BA, Wardell CP, Melchor L, et al. Intraclonal heterogeneity and distinct molecular mechanisms characterize the development of t(4;14) and t(11;14) myeloma. Blood 2012;120:1077–86.
[56] Ayliffe MJ, Behrens J, Stern S, Sumar N. Association of plasma cell subsets in the bone marrow and free light chain concentrations in the serum of monoclonal gammopathy patients. J Clin Pathol 2012;65:758–61.
[57] Keats JJ, Chesi M, Egan JB, et al. Clonal competition with alternating dominance in multiple myeloma. Blood 2012;120:1067–76.
[58] Kuhnemund A, Liebisch P, Bauchmuller K, et al. 'Light-chain escape-multiple myeloma'—an escape phenomenon from plateau phase: report of the largest patient series using LC-monitoring. J Cancer Res Clin Oncol 2009;135:477–84.
[59] Zamarin D, Giralt S, Landau H, et al. Patterns of relapse and progression in multiple myeloma patients after auto-SCT: implications for patients' monitoring after transplantation. Bone Marrow Transplant 2013;48:419–24.
[60] Abraham RS, Katzmann JA, Clark RJ, Bradwell AR, Kyle RA, Gertz MA. Quantitative analysis of serum free light chains. A new marker for the diagnostic evaluation of primary systemic amyloidosis. Am J Clin Pathol 2003;119:274–8.
[61] Akar H, Seldin DC, Magnani B, et al. Quantitative serum free light chain assay in the diagnostic evaluation of AL amyloidosis. Amyloid 2005;12:210–5.
[62] Bochtler T, Hegenbart U, Heiss C, et al. Evaluation of the serum-free light chain test in untreated patients with AL amyloidosis. Haematologica 2008;93:459–62.
[63] Katzmann JA, Abraham RS, Dispenzieri A, Lust JA, Kyle RA. Diagnostic performance of quantitative kappa and lambda free light chain assays in clinical practice. Clin Chem 2005;51:878–81.
[64] Palladini G, Russo P, Bosoni T, et al. Identification of amyloidogenic light chains requires the combination of serum-free light chain assay with immunofixation of serum and urine. Clin Chem 2009;55:499–504.
[65] Nasr SH, Valeri AM, Cornell LD, et al. Renal monoclonal immunoglobulin deposition disease: a report of 64 patients from a single institution. Clin J Am Soc Nephrol 2011;7:231–9.
[66] Wechalekar AD, Lachmann HJ, Goodman HJB, Bradwell AR, Hawkins PN. Role of serum free light chains in diagnosis and monitoring response to treatment in light chain deposition disease. Haematologica 2005;90:PO1414a.
[67] Hutchison CA, Cockwell P, Stringer S, et al. Early reduction of serum-free light chains associates with renal recovery in myeloma kidney. J Am Soc Nephrol 2011;22:1129–36.
[68] Hutchison CA, Batuman V, Behrens J, et al. The pathogenesis and diagnosis of acute kidney injury in multiple myeloma. Nat Rev Nephrol 2011;8:43–51.
[69] Basnayake K, Hutchison C, Kamel D, et al. Resolution of cast nephropathy following free light chain removal by haemodialysis in a patient with multiple myeloma: a case report. J Med Case Reports 2008;2:380.

[70] Basnayake K, Cheung CK, Hutchison C, et al. Differential progression of renal scarring and determinants of late renal recovery in sustained dialysis dependent acute kidney injury secondary to myeloma kidney. Presented at BRS/RA Conference; 2010. p. P239a.
[71] Hutchison CA, Cockwell P, Reid S, et al. Efficient removal of immunoglobulin free light chains by hemodialysis in multiple myeloma: in vitro and in vivo studies. J Am Soc Nephrol 2007;18:886–95.
[72] Leung N, Gertz MA, Zeldenrust SR, et al. Improvement of cast nephropathy with plasma exchange depends on the diagnosis and on reduction of serum free light chains. Kidney Int 2008;73:1282–8.
[73] Pillon L, Sweeting RS, Arora A, et al. Approach to acute renal failure in biopsy proven myeloma cast nephropathy: is there still a role for plasmapheresis? Kidney Int 2008;74:956–61.
[74] Shum HP, Chan KC, Chow CC, Kho BC, Yan WW. Cast nephropathy with acute renal failure treated with high cut-off haemodialysis in a patient with multiple myeloma. Hong Kong Med J 2010;16:489–92.
[75] Hutchison CA, Bradwell AR, Cook M, et al. Treatment of acute renal failure secondary to multiple myeloma with chemotherapy and extended high cut-off hemodialysis. Clin J Am Soc Nephrol 2009;4:745–54.